

Supporting Information

Structural Features of the Cytochrome c Molten Globule Revealed by Fluorescence Energy Transfer Kinetics.

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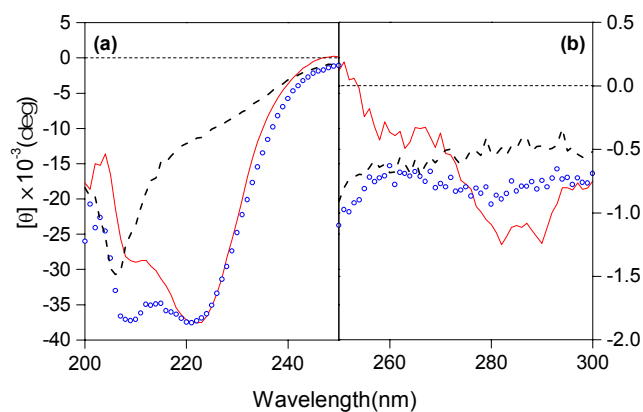


Figure S1. (a) Far- and (b) near-UV CD spectra of DNS(C102)-cyt *c* at 22 °C. (—) the native state, (o) A state, and acid-denatured state (- -).

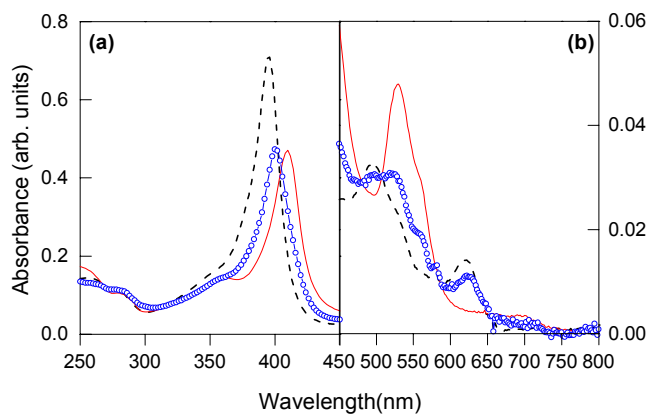


Figure S2. (a) Soret and (b) Q-band absorption spectra of DNS(C102)-cyt *c* at 22 °C. (—) the native state, (o) A state, and acid-denatured state (- -).

Experimental Section

DNS(C102)-cyt *c* and a model compound (*N*-acetylcysteine derivative of ((acetyl)amino)ethyl)amino)naphthalene-1-sulfonic acid, AEDANS) were prepared as previously described.¹³ Circular dichroism data were acquired using an Aviv Model 62DS spectropolarimeter equipped with a thermostated sample holder. Spectra were collected at 22 °C unless specified. Buffers used were: 0.05 M sodium phosphate, pH 7 for the native protein; H₂SO₄, pH ~ 2 for the acid-unfolded state; 0.7 – 1 M Na₂SO₄/H₂SO₄, pH ~2 for the molten globule state. For the thermal unfolding transition of the DNS(C102)-cyt *c* molten globule, the ellipticity at 222 nm was recorded from –1 °C to 80 °C at 1 °C intervals (0.7 M Na₂SO₄/H₂SO₄, pH ~2, [DNS(C102)-cyt *c*] = 2.0 – 10.0 μM). The time between successive data points was about 8 min. Reversibility was confirmed by running the reaction in reverse back to the initial temperature or by cooling the sample to the initial temperature, revisiting a few data points, and comparing the ellipticity to the prior value. Fluorescence decay measurements were performed using the third harmonic of regeneratively amplified, mode-locked Nd-YAG laser (355 nm, 50 ps, 0.5 mJ) for excitation and a picosecond streak camera (Hamamatsu C5680) for detection. Magic-angle excitation and collection conditions were employed throughout. DNS fluorescence was selected with a long-pass cutoff filter (>430 nm). The C5680 was used in photon counting mode. FET kinetics data were analyzed as described previously.¹³